Chloroform Fumigation–Incubation: Is it the Same Old Thing?

Alan Franzluebbers

Watkinsville, GA

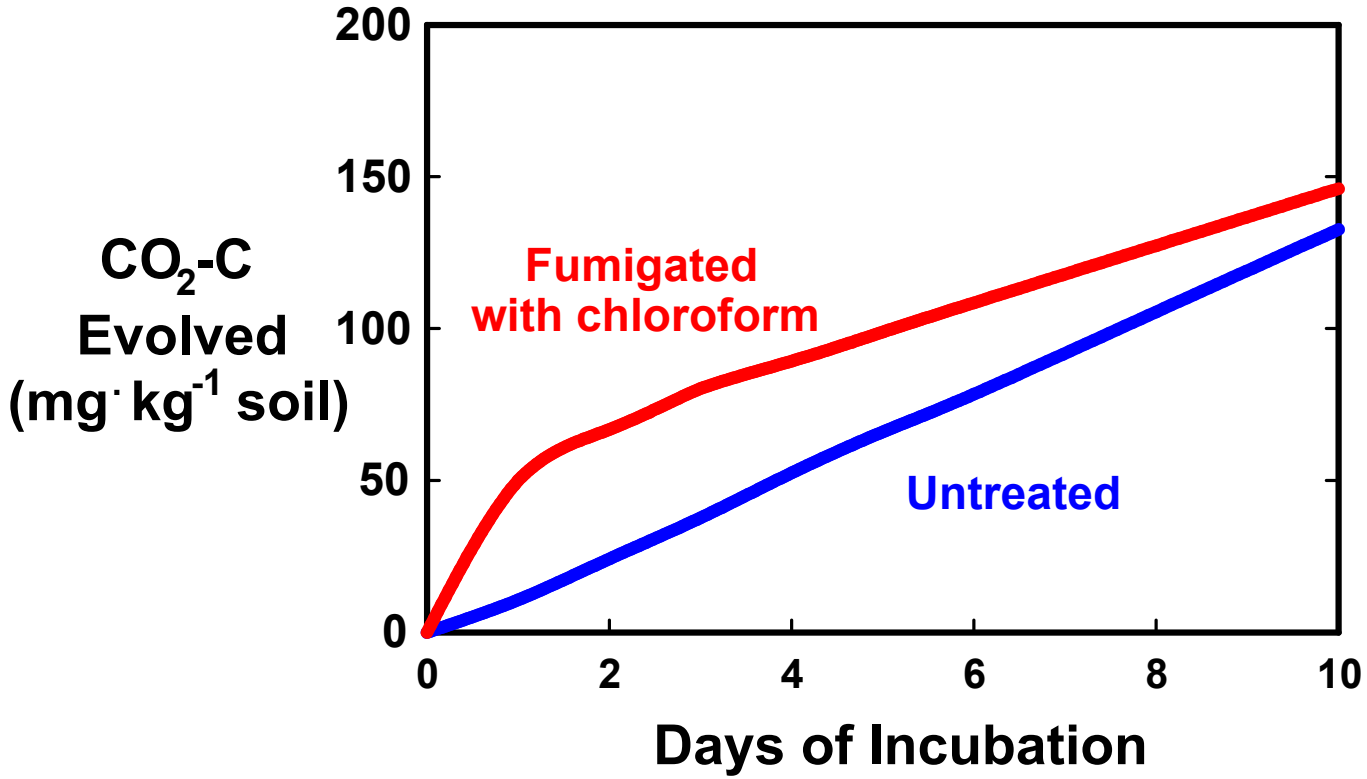
Rick Haney
Frank Hons

College Station, TX

$CHCl_3$ Fumigation–Incubation

Respiratory response during 10 d following fumigation is the result of decomposition of killed microorganisms

^ the flush is related to soil microbial biomass (SMB)



$CHCl_3$ Fumigation–Incubation

Advantages

- Yields holistic estimate of microbial community
- Obtain wide diversity of elemental concentrations of SMB
- Can obtain isotopic signatures
- Estimates of SMB related with other methodologies
- Relatively simple methodology
- Inexpensive equipment required
- Has been assessed on a wide diversity of soils
- Allows incubation to overcome unknown extraction efficiencies

$CHCl_3$ Fumigation–Incubation

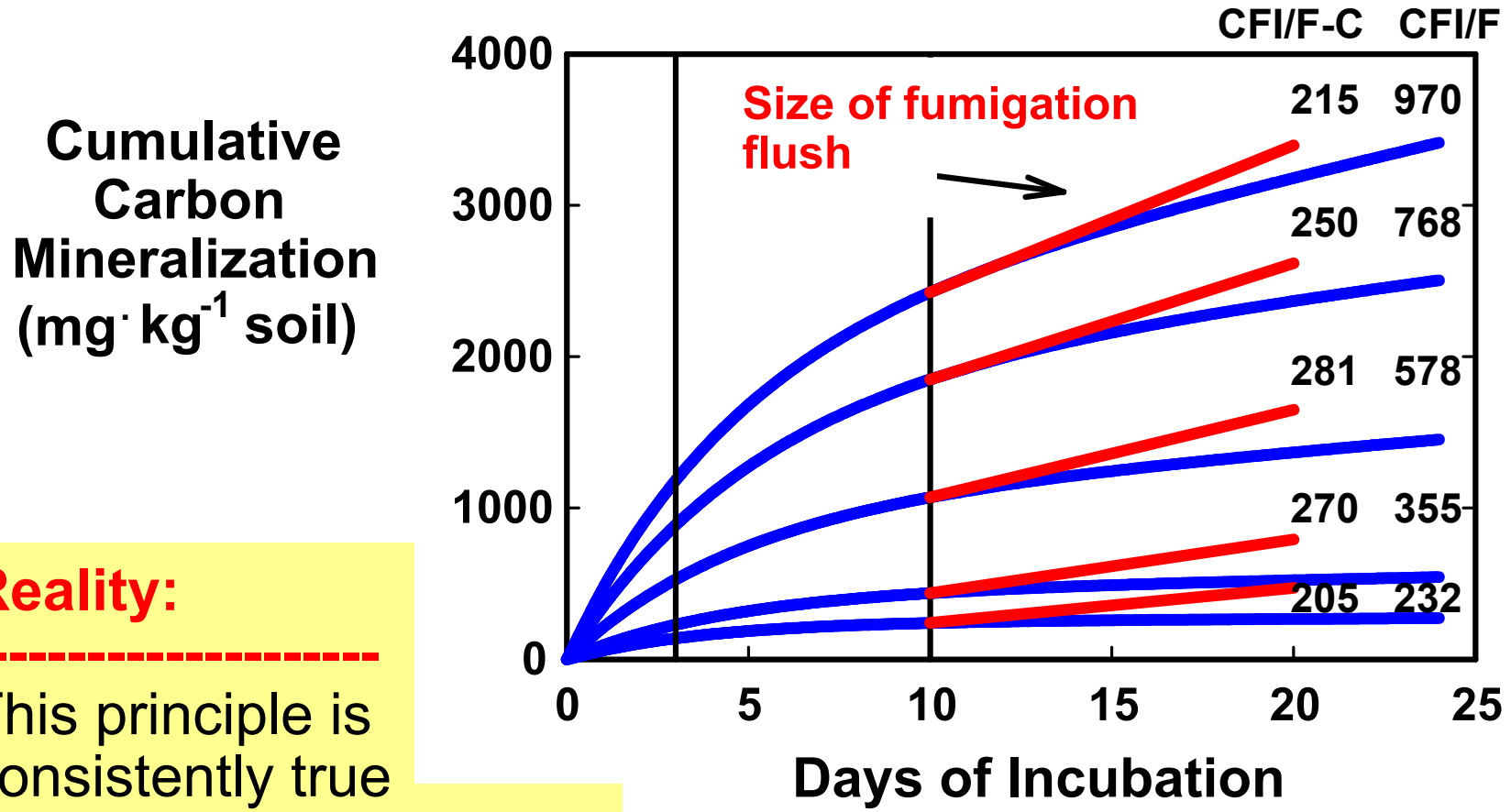
Why isn't CFI more universally utilized ?

- Long-standing controversy over "control issue"

  - Original method of Jenkinson (1966) calculated SMB as:
    SMB = [CO_2-C (0-10 d fumigated) – CO_2-C (0-10 d control)] / k_C
  - Lower respiration during 10-20 d compared with 0-10 d of control sample led Jenkinson & Powlson (1976) to suggest:
    SMB = [CO_2-C (0-10 d fumigated) – CO_2-C (10-20 d control)] / k_C
  - Lower respiration of fumigated sample following the subsidence of flush compared with the control sample led Chaussod & Nicolardot (1982) to suggest:
    SMB = [CO_2-C (0-10 d fumigated) – CO_2-C (10-20 d fumigated)] / k_C
  - Voroney & Paul (1984) suggested no control was needed after investigating the fate of ^{14}C-labelled glucose in SMB:
    SMB = [CO_2-C (0-10 d fumigated)] / k_C

$CHCl_3$ Fumigation–Incubation

Principle:
Soils high in potential microbial activity have high SMB

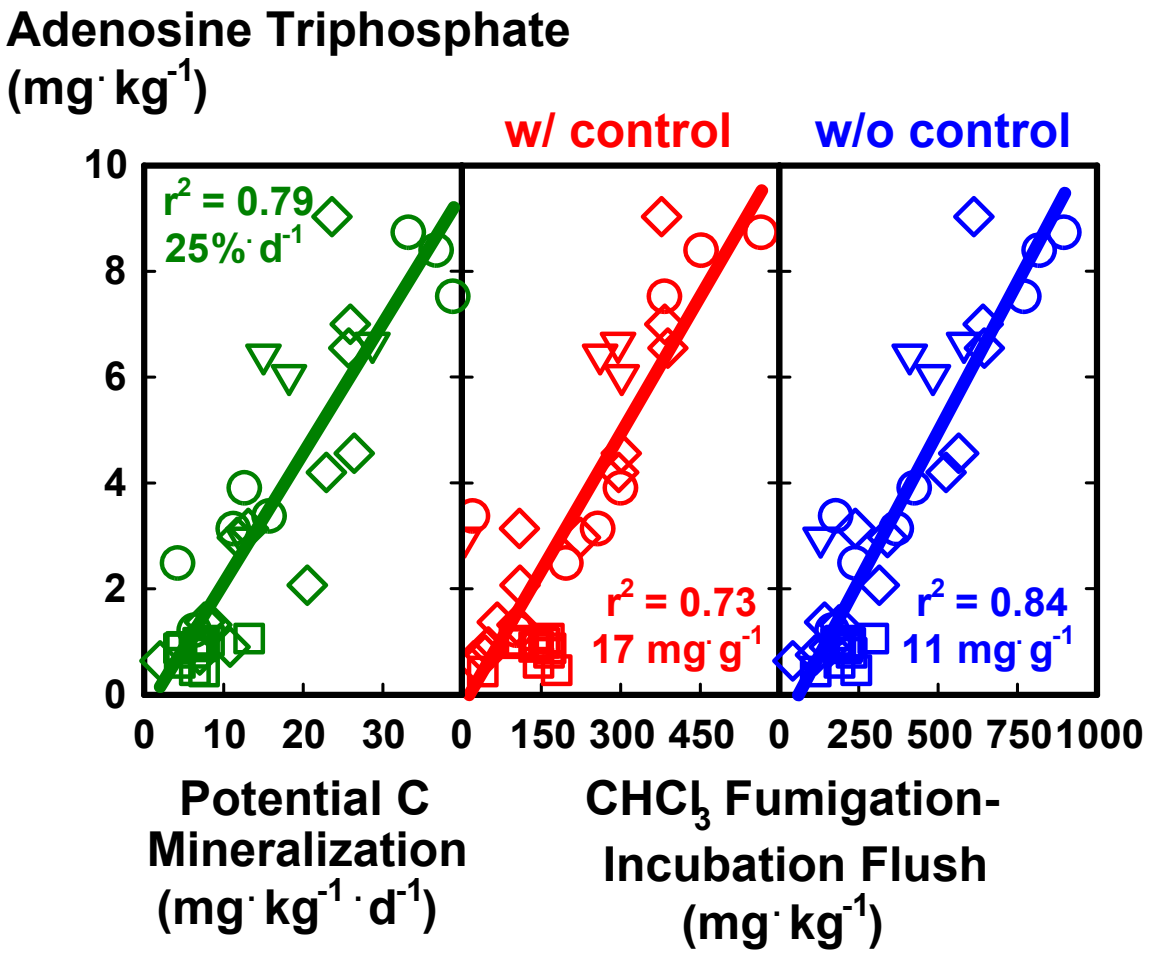


Reality:

This principle is consistently true only with a modification of the original method, i.e. by not subtracting a control.

$CHCl_3$ Fumigation–Incubation

Argument:
Microbial activity is not the same as microbial biomass



Jenkinson et al. (1979)
Oades and Jenkinson (1979)
Sparling (1981)
Vance et al. (1987a)

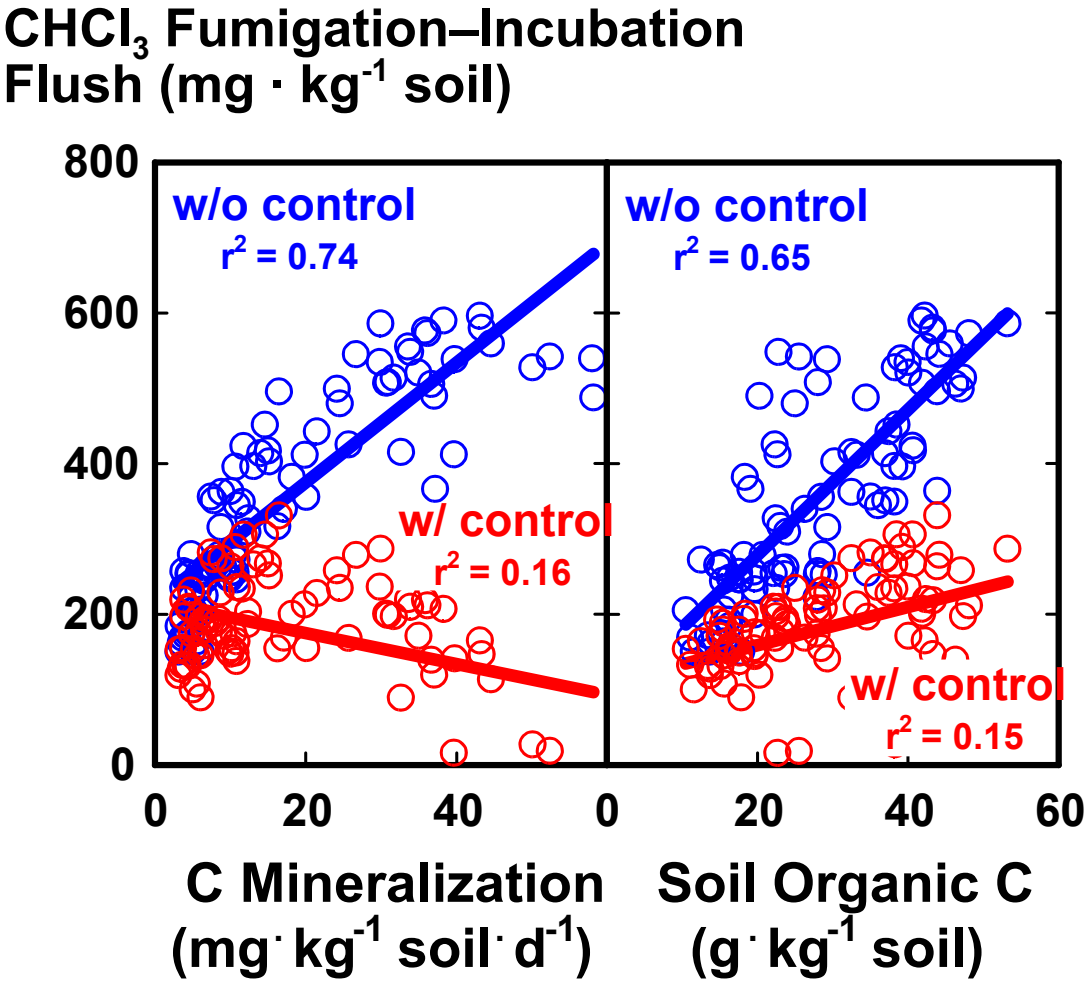
Technically true,

but potential microbial activity is a reflection of the resources available for microorganisms.

A strong relationship between potential C mineralization and ATP independently corroborates this relationship.

$CHCl_3$ Fumigation–Incubation

Why subtract a control,
when it results in unreasonable estimates of SMB ?



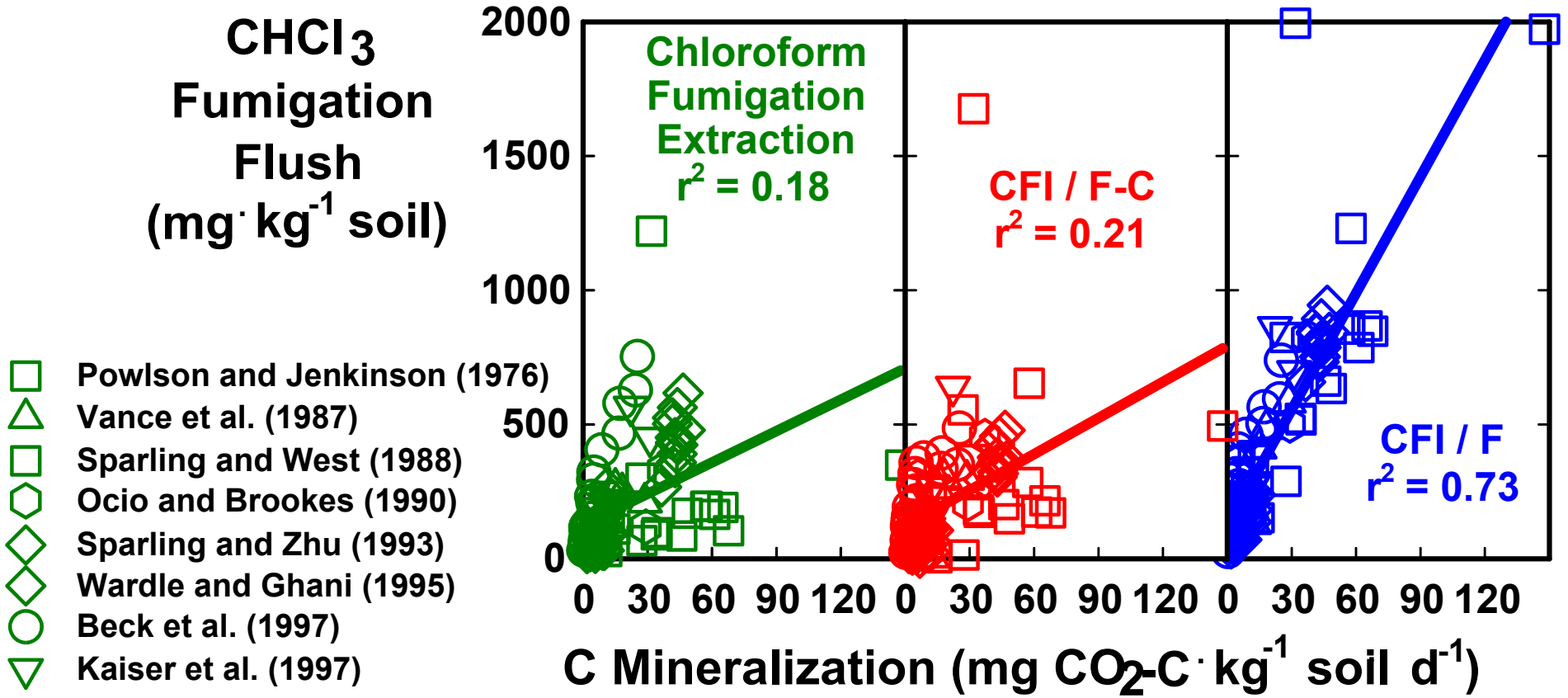
Soil microbial biomass should be related to substrate availability (i.e. SOC) and utilization (i.e. potential C mineralization).

CFI without a control is more consistently related with relevant soil organic matter pools.

From Franzluebbers et al. (1999) Soil Biol. Biochem. 31:395-405.

$CHCl_3$ Fumigation–Incubation

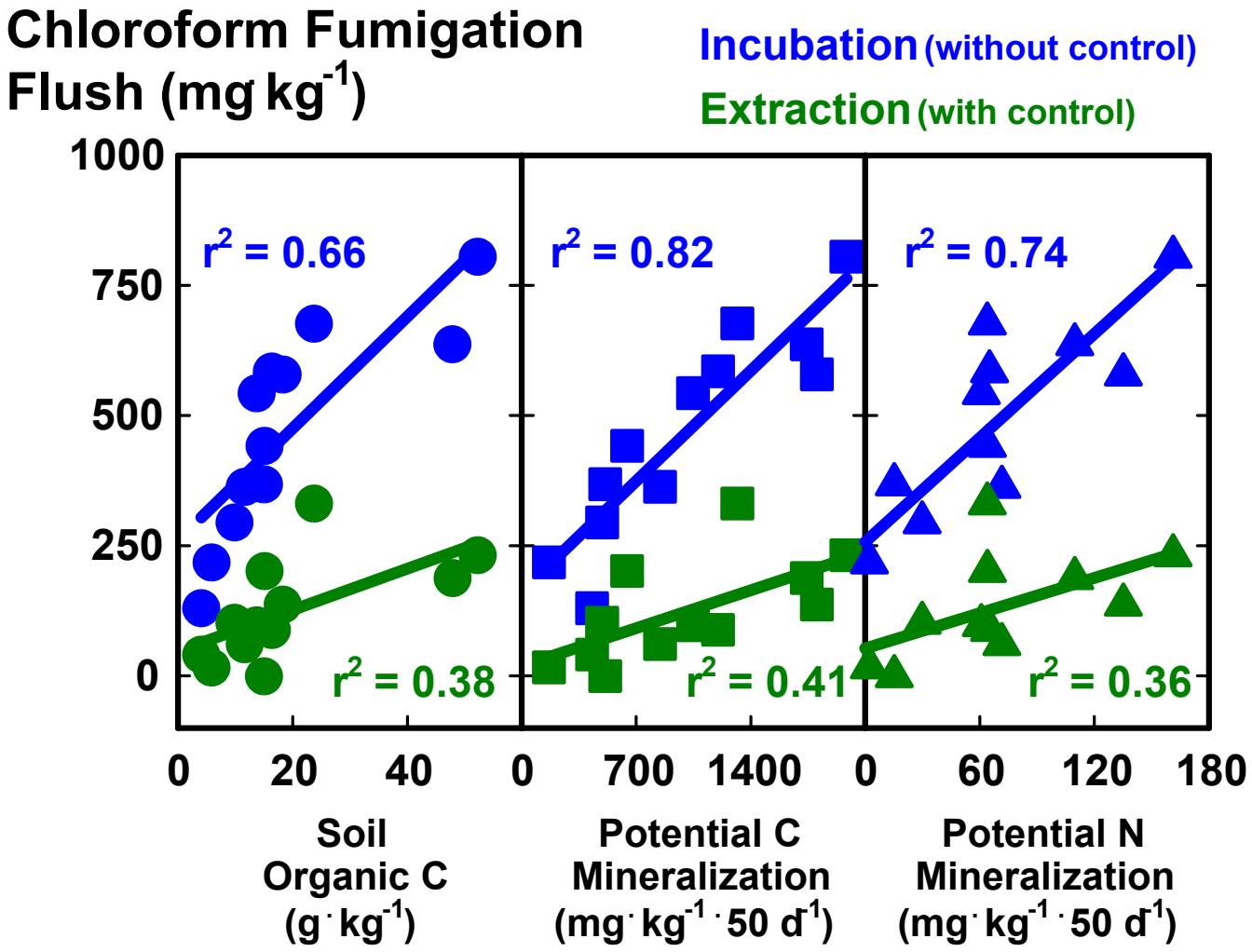
Why use an "old" method ?



Data from different soils support CFI without a control.

$CHCl_3$ Fumigation–Incubation

Why use an "old" method ?



Because

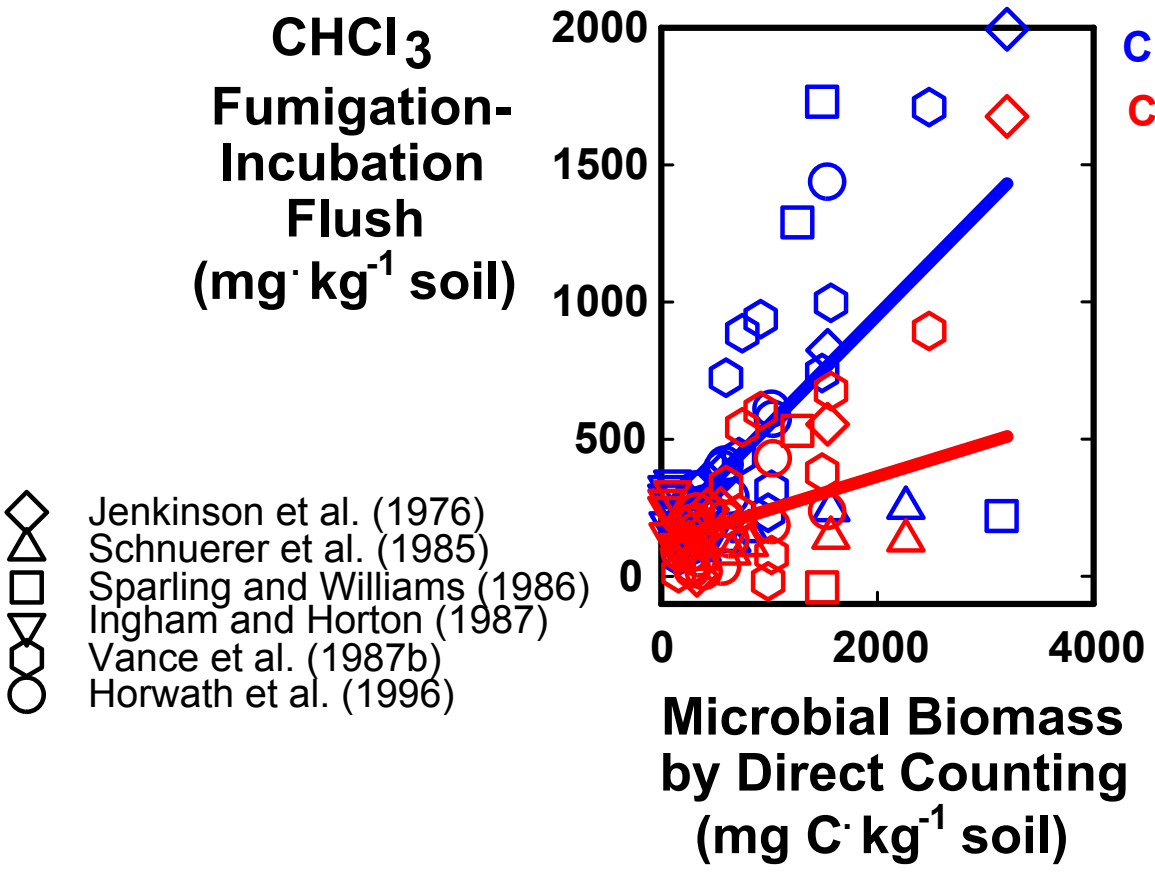
(1) it relates well to substrate availability and utilization

(2) it is more consistent than other proposed methods

From Haney et al. (2001) Soil Biol. Biochem. 33:1501-1507.

$CHCl_3$ Fumigation–Incubation

"The effect of omitting the control of course, is to apparently measure a very much larger (up to twice as large) pool of biomass than if the control is subtracted, as in the original method"



CFI / F = 152 + 0.40(direct), r^2 = 0.43

CFI / F-C = 122 + 0.12(direct), r^2 = 0.08

CFI / F

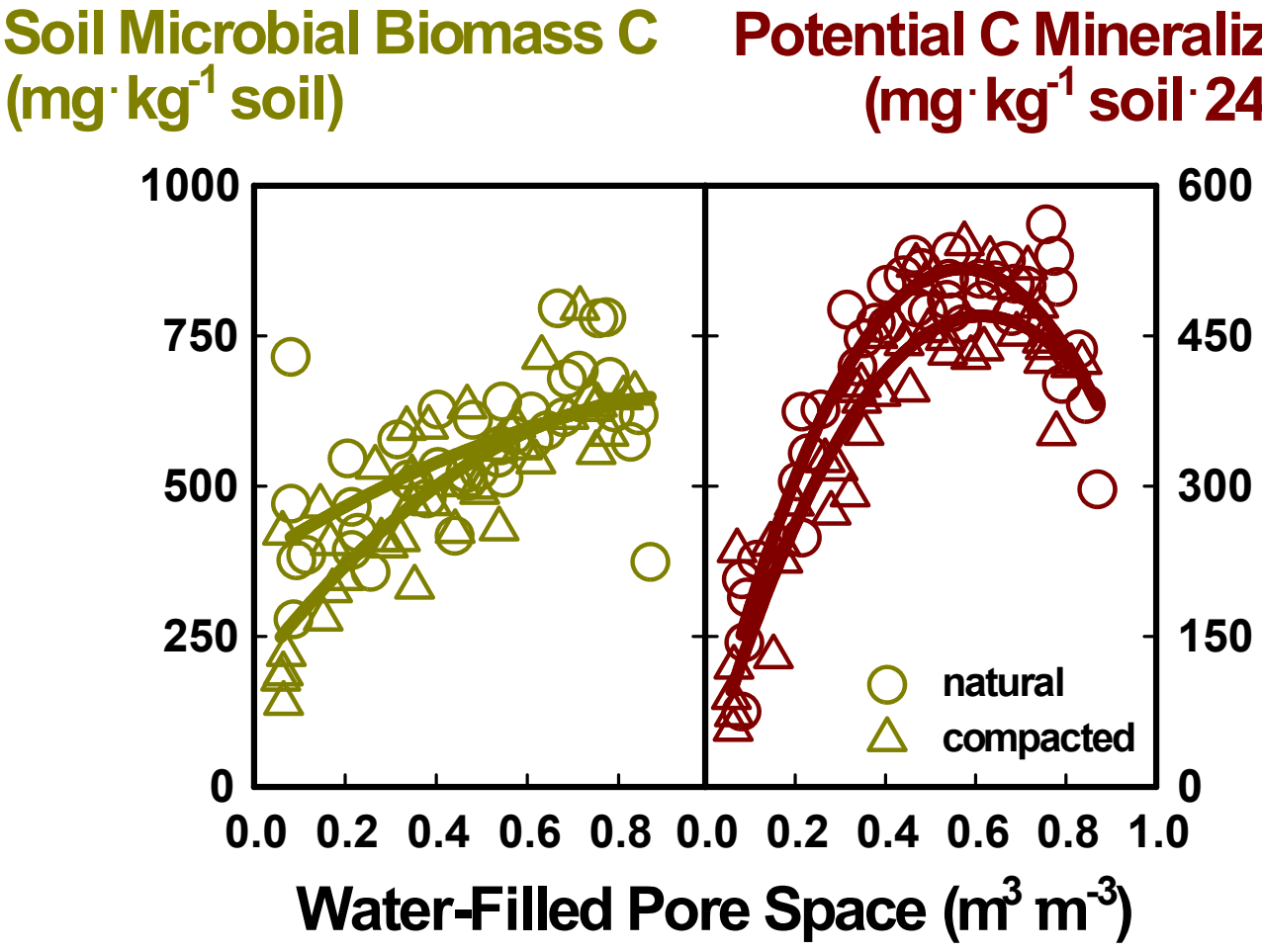
SMB = C_f / k_C

CFI / F-C

SMB = [C_f – C_{uf}] / k_C

Exploring Microbial Biomass w/ CFI

Effect of water on soil microbial biomass and activity



From Franzluebbers (1999) Appl. Soil Ecol. 11:91-101.

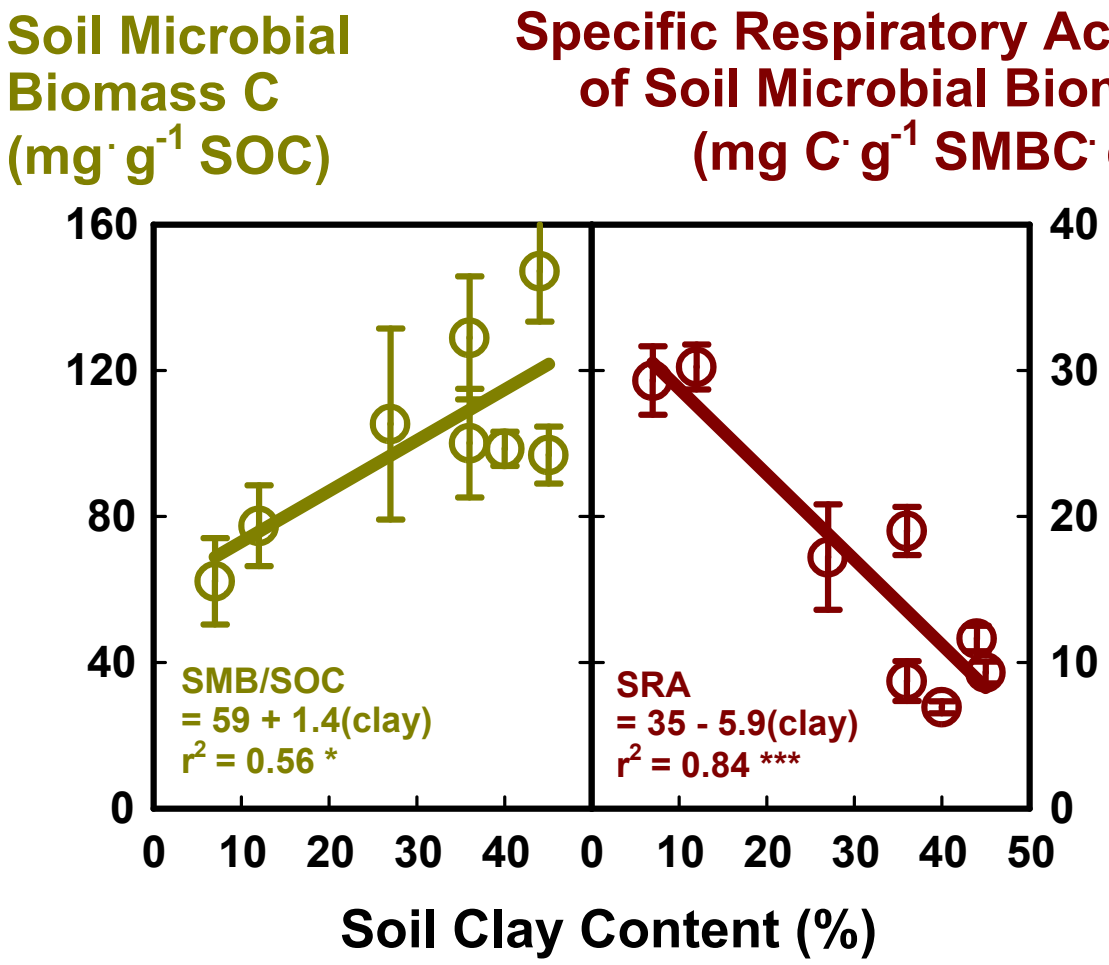
Investigative value

Although responsive to WFPS, estimates of soil microbial biomass C did not fluctuate as much as would be expected with soil microbial activity.

Soil compaction limited microbial biomass at low WFPS.

Exploring Microbial Biomass w/ CFI

Soil textural effects on microbial biomass



Investigative value

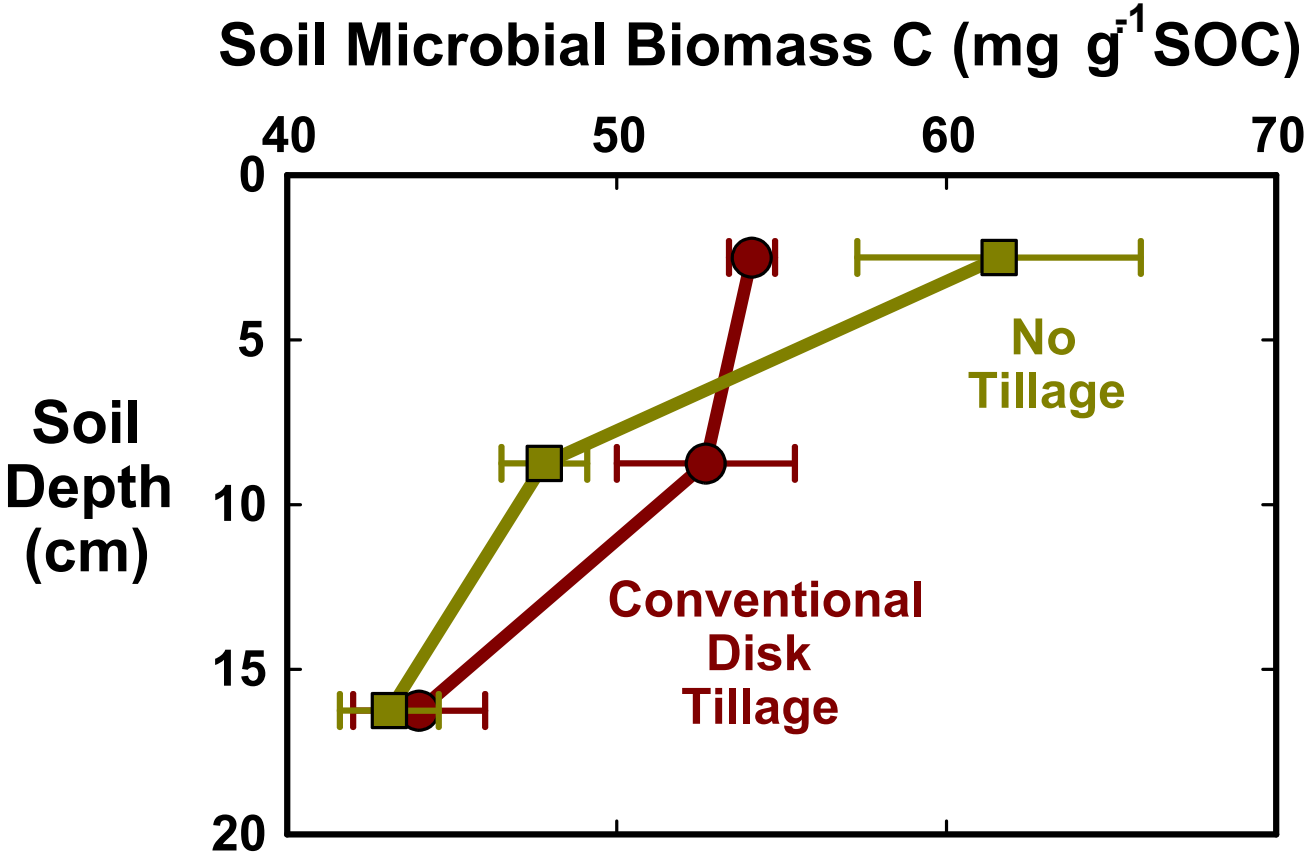
Soil microbial biomass increased with increasing clay content (reduced water fluctuations or protection from faunal grazing).

Specific respiratory activity decreased with increasing clay content (+ reduced substrate availability or isolation of SMB).

From Franzluebbers et al. (1996) Soil Biol. Biochem. 28:1367-1372.

Exploring Microbial Biomass w/ CFI

Depth distribution of soil microbial biomass under different tillage systems



From Franzluebbers et al. (1995) Soil Sci. Soc. Am. J. 59:460-466.

Investigative value

If SMB responded exactly the same as SOC, then there would be no difference in SMB/SOC with depth or tillage.

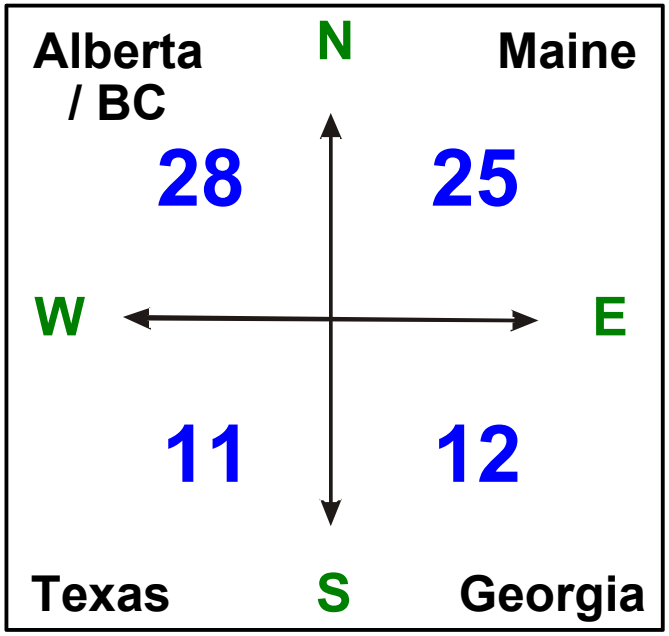
SMB was enriched at depths of residue placement [i.e. at surface of NT and within the tillage zone (0-12 cm) of CT].

Exploring Microbial Biomass w/ CFI

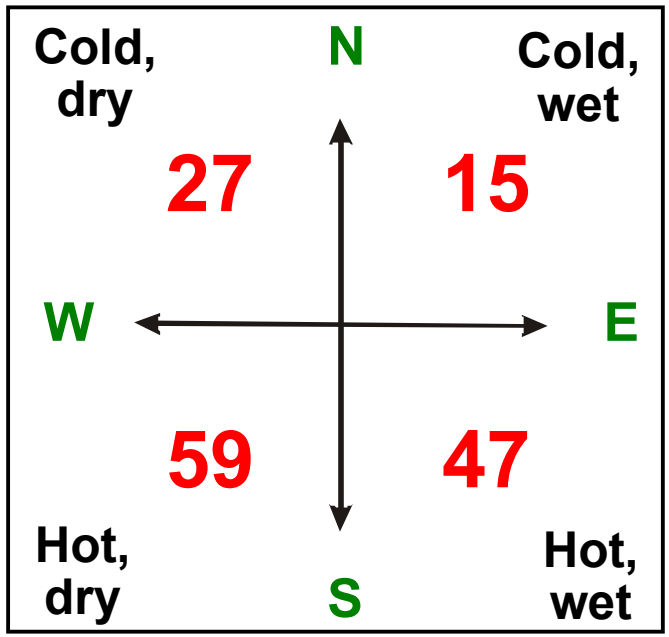
Effect of macroclimatic variables on soil microbial biomass

In North America

Soil Organic C
(mg g^{-1} soil)



Soil Microbial Biomass C
(mg g^{-1} SOC)



From Franzluebbers et al. (2001) Soil Biol. Biochem. 33:1103-1111.

Investigative value

Although soil organic C is greater in cold than in hot regions, the fraction of C that is SMB is greater in hot regions.

Dry regions also support a larger fraction of SMB than wet regions.

$CHCl_3$ Fumigation–Incubation

Conclusions

- The “control issue” obscured the value of chloroform fumigation–incubation
- Incubation is a preferred analytical tool that allows soil organisms to express themselves within the confines of their environment
- Equipment and labor resources needed are low
- Precision of SMB estimates is high
- Accuracy, as with all SMB methods, is relatively unknown
- Wide range of ecological studies with meaningful results can be obtained with chloroform fumigation–incubation